Loss of KLF10 expression does not affect the passive properties of single myofibrils

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1. Introduction

KLF10 is a member of the Krüppel-like family of transcription factors (Subramaniam et al. 1995) that plays important roles in the musculoskeletal system (Kammoun et al. 2016). Although KLF10 is strongly expressed in skeletal muscle, its role in muscle function and pathology has not been fully elucidated. Previous studies have shown structural changes such as muscle hypertrophy, a switch from oxidative to glycolytic fibers and major changes in the global mitochondrial mass in the soleus and extensor digitorum longus (EDL) muscles of KLF10 knock-out (KO) mice (Kammoun et al. 2020). We demonstrated that the loss of KLF10 expression elicited muscle-type specific effects on the passive properties of fibers (Kammoun et al. 2016). While KLF10 KO soleus fibers showed an increase in passive force, KLF10 KO EDL fibers showed a decrease in passive force at corresponding sarcomere lengths, compared to the control wild type (WT) fibers. However, it is not clear whether these adaptations in the passive properties of KFL10 KO fibers originate within sarcomeres or outside sarcomeres. Titin is a molecular spring inside sarcomeres that spans each half-sarcomere from the Z-line to the M-line and is known to produce most of the passive force in isolated sarcomeres and myofibrils (Granzier et al. 2000). Titin isoforms vary across muscles and account for the differences in passive force between myofibrils harvested from different muscles (Horowits et al. 1992). The present study for the first time has performed multi-scale analysis to elucidate the impact of KLF10 gene on passive mechanical properties of muscle. Thus, the purpose of this study was to gain insight into the origin of the passive behavior observed at the fiber scale and at the myofibril (titin) scale.

2. Methods

2.1 Animals

Three-month-old littermate female animals derived from heterozygous breeding were used in this study. All mice were maintained in a temperature controlled room (22 ± 2 °C) with light/dark cycle of 12 hours. Animals had free access to water and were fed with standard laboratory chow ad libitum. The protocol was approved by the French ministry of higher education, research and innovation (DUO-4776).

2.2 Sample preparation

Twelve soleus muscles were harvested from 6 WT mice and 6 KLF10 KO mice and 10 EDL muscles were harvested from 5 WT mice and 5 KLF10 KO mice. Subsequently the muscles were kept for 12 hours at 4 °C in a skinning solution (70 mM potassium propionate, 8 mM magnesium acetate, 5 mM EGTA, 7 mM ATP, 6 mM Imidazole, 10 mM PMSF,
50 $mg.L^{-1}$ Trypsin inhibitor, 4 $mg.L^{-1}$ Leupeptine, pH = 7.1). Muscles were washed in a series of graded glycerol concentrations (12.5, 25 and 50 %) in the skinning solution prior to storage in a 50/50 glycerol/skinning solution at -20 °C.
Myofibrils isolated from WT and KFL10 KO soleus and EDL muscles were fixed under an inverted microscope to a glass needle attached to a length controller at one end, and to a nanolever at the other end, allowing for length changes and force measurements, respectively (Joumaa et al. 2008). The striation pattern of the myofibrils was projected onto a linear photodiode array for determination of individual sarcomere lengths. The diameter of the myofibrils was measured at a magnification of 40X, and used to determine the cross-sectional area of the myofibril.

2.3 Testing protocol

Myofibrils were set at an average sarcomere length of 2.4 µm. They were then passively stretched at a speed of 0.1µm/s to an average sarcomere length of 3.4 µm. The stretch was held for 20 seconds until a steady-state force was reached, and then released. Passive force reached at steady-state was determined and converted to stress by dividing force by the cross-sectional area of the myofibril.

2.4 Titin protein

The molecular weight of the titin protein in WT and KLF10 KO soleus and EDL muscles was determined

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using 2 % agarose-strengthened SDS polyacrylamide gels with a Laemmli buffer system (Joumaa et al. 2008). Gels were run at a constant voltage of 22 V overnight at room temperature. Rabbit psoas muscle, which expresses two titin isoforms with molecular weights of 3295 and 3416 kDa (Prado et al. 2005), was used as migration standards.

2.5 Statistical analysis

The SystatTM V11 (Systat Software Inc., CA, USA) was used and non parametric two-sample Mann-Whitney tests were utilized to compare the diameters and the passive stress between the soleus and EDL myofibrils as a function of genotype. Results were considered significant for $p < 0.05$.

3. Results and discussion

Figure 1 shows the reponse of WT and KLF10 KO soleus myofibrils to the stretching protocol.
There was no significant difference in passive stress between the WT and the KLF10 KO myofibrils for both soleus and EDL myofibrils (Table 1).

	Passive Stress (nN/µm^2)	
	WT	KLF10 KO
Soleus (n=11)	52 ± 20	48 ± 24
EDL (n=11)	42 ± 14	42 ± 23

Table 1. Mean steady-state stress (± SD) at a sarcomere length of 3.4 µm in WT and KFL10 KO soleus and EDL myofibrils.

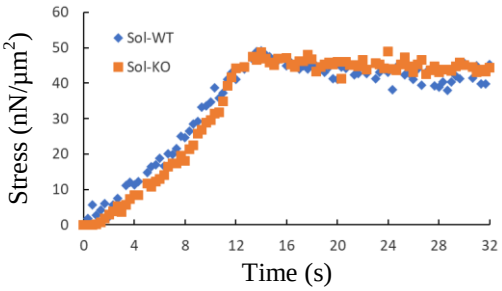

Figure 1. The mechanical responses of WT (blue) and KFL10 KO (orange) soleus myofibrils when passively stretched from a sarcomere length of 2.4 µm to 3.4 µm. The same behavior is obtained from WT and KFL10 KO EDL myofibrils.

There was no difference in titin mass measured in WT and KFL10 KO soleus and EDL muscles (Table 2).

	Titin Mass (kDa)	
	WT	KLF10 KO
Soleus (n-6)	3567 ± 17	3561 ± 17
EDL (n=5)	3533 ± 22	3535 ± 21

Table 2. Mean titin molecular mass (± SD) in WT and KFL10 KO soleus and EDL muscles.

The present study demonstrates that the absence of KLF10 gene does not impact the passive properties of soleus and EDL myofibrils or titin molecular weight. As a consequence, the changes in the passive properties found at the fiber level do not originate from sarcomeric structures. In addition, the result of the molecular weights suggest that there is no difference in titin isoforms between WT and KLF10 KO mice.

4. Conclusions

We conclude from the results of this study that the observed fibre-type specific changes in passive force in KLF10 KO mice muscles are not caused by sarcomere intrinsic structures but must originate outside the sarcomeres, likely in the collagen-based extracellular matrix.

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